

OPTIMIZATION OF POLYPHENOLS ENCAPSULATION FROM ORTHOSIPHON STAMINEUS

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ABSTRACT

Orthosiphon stamineus, *O. stamineus*, (vernacular name: ‘misai kucing’), is a traditional herb that can be found grows in Southeast Asia. *O. stamineus* is a herbal shrub with well-developed creeping rootstock. The leaves are simple, green, and arranged in opposite pairs. *O. stamineus* is used as traditional remedy for joint inflammation, gout, diabetes, oedema, eruptive fever, jaundice, arthritis, rheumatism diuresis, hepatitis, urinarylithiasis, influenza hypertension, and kidney stones. The phenolic compounds content in *O. stamineus* as antioxidant source is proven there. There are some research done about microencapsulation of polyphenols from *O. stamineus*, however, there is not much optimization protocol has been established for phenolic extraction and microencapsulation from *O. stamineus*. Hence, the aim of this study is to optimize the polyphenol extraction from *O. stamineus* by studying the effects of temperature on the recovery of phenolic compounds in spray dryer, and total solid percentage and the maltodextrin/whey protein mix in microencapsulation. UPLC is used to analyze moisture, total solid and total flavonoid content. The optimization on parameters such as temperature, total solid content and ratio of microencapsulation material shall be done using Response Surface Modelling (RSM) and some other software like Design-Expert and Microsoft Excel. This research project is expected to deliver the least phenolic content degradation based on parameters involved.

ABSTRAK

Orthosiphon stamineus, *O. stamineus*, (nama vernakular: ‘misai kucing’), adalah sejenis herba tradisional yang dapat ditemui di Asia Tenggara. *O. stamineus* adalah pokok herba renek yang mempunyai akar yang menjalar. Daunnya berwarna hijau dan bersusun pada arah yang bertentangan. *O. stamineus* digunakan secara tradisional sebagai penawar untuk keradangan pada sendi, gout, diabetes, edema, demam panas, penyakit kuning, artritis, penyakit sendi diuresis, hepatitis, urinarylithiasis, darah tinggi, influenza, dan juga batu karang. Kandungan fenolik sebagai bahan antioksidan telah terbukti terdapat dalam tumbuhan ini. Terdapat beberapa kajian berkenaan pemikroenkapsulan polifenol daripada *O. stamineus*. Namun begitu, kaedah untuk mengoptimumkan kaedah pemikroenkapsulan pengekstrakan fenol daripadanya. Oleh yang demikian, kajian ini dijalankan dengan tujuan mengoptimumkan pengekstrakan polifenol daripada *O. stamineus* dengan meneliti aspek kesan suhu pada pemerangkapan kandungan polifenol di dalam pengering semburan, dan peratusan jumlah pepejal serta kandungan campuran isolat whey protein dan maltodekstrin di dalam pemikroenkapsulan. UPLC pula digunakan untuk menganalisis kelembapan, jumlah pepejal, dan jumlah kandungan flavonoid. Pengoptimuman terhadap pembolehubah parameter seperti suhu, peratusan jumlah pepejal serta kandungan campuran di dalam pemikroenkapsulan akan dilakukan menggunakan RSM dan perisian-perisian lain termasuk Design Expert dan juga Microsoft Excel. Kajian ini diharapkan untuk memberi degradasi kandungan fenolik yang paling minimum berdasarkan pembolehubah parameter yang telah disebutkan.

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LIST OF ABBREVIATIONS

O. stamineus	Orthosiphon Stamineus
UPLC	Ultra-Performance Liquid Chromatography
RSM	Response Surface Modelling
WPI	Whey Protein Isolate
MD	Maltodextrin
RA	Rosmarinic Acid
SIN	Sinensetin
EUP	Eupatorin

1 INTRODUCTION

1.1 Motivation and statement of problem

Orthosiphon stamineus , Benth (Lamiaceae) or Cat's Whisker is well known as 'Misai kucing' by the locals in Malaysia. This handful herb can be found in South East Asia such as Malaysia, Indonesia, Thailand and Philippines. This plant is commonly used traditionally by the medicine practitioners for the treatment of joint inflammation. Other than that, *O. stamineus* is also used for the treatment of gout, arthritis, rheumatism and remedy for kidney stones. Commercially, *O. stamineus* is famous because of its slimming property. *O. stamineus* is known as Java Tea in the market for its safe and effective mild herbal diuretic that throws out excess fluids, nitrogen substances and sodium chloride (Pouralinazar et al, 2012). Pharmacological effects of *O. stamineus* are attributed to presence of polyphenolics, glycosides, lipophilic flavones, rosmarinic acid (RA) and caffeic acid derivatives, triterpenes, and diterpens. The lipophilic flavones of *O. stamineus* including sinensetin (SIN), eupatorin (EUP) and 3'-hydroxy-5, 6, 7, 4' tetramethoxyflavone (TMF) have been given considerable interest as markers of pharmacological activity by several researchers (Aisha, Majid, & Ismail, 2014).

1.2 Ultrasonic-assisted extraction

This extraction method is an easy and highly feasible method for extraction of *O. stamineus* and the operation can be applied rapidly in most solvents for large-scale preparations equipped for industrial purposes. This extraction method also favored and fetches interests of many when dealing with extraction from plant material in pursuing bioactive substance content.

1.3 Microencapsulation

Microencapsulation is one technique proposed to preserve flavonoid content from degrading. Microencapsulation is one of the methods to preserve the antioxidant properties and the flavonoid content in *O. stamineus*. Microencapsulation technique is an effective way to protect the food ingredients from being deteriorated or having any

volatile losses. Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating, or embedded in a homogeneous or heterogeneous matrix which providing a physical barrier between the core compound and the other components of the product (Ling, 2013). Microencapsulation is defined as a process in which small particles are enclosed by a coating, or embedded in a homogeneous or heterogeneous matrix by wall material or encapsulating agent. The wall material selection also a main concerns for microencapsulation process.

1.4 Spray Drying

Spray drying is an important method used by the food industries in the production of microencapsulated flavors to improve handling and dispersion properties. For decades, spray drying has been applied to encapsulate food ingredients such as carotenoids, flavors and lipids. During the drying process, the evaporation of solvent, and the entrapment of the favored compound occurs instantaneously.

1.5 Response Surface Modelling (RSM)

Response surface modelling (RSM) as an effective statistical tool can be used for investigating the influences of various variables, affecting the responses (Division, 2002). The interactions of variables in this optimization technique affecting the dependent variables are considered accurately. Many parameters could be affecting the response. Therefore it is essential to choose the best statistical model so that the number of experiments can be minimized, as well as evaluating the effects of important variables and interactions among them in multivariable system.

1.6 Objectives

The following are the objectives of this research:

- To minimize thermal degradation of *O. stamineus* compound by spray drying and microencapsulation method.
- To develop spray drying process to convert the extract *O. stamineus* into powder solid form.
- To perform microencapsulation technique by encapsulating the bioactive compounds in *O. stamineus* extract using microencapsulating agent such as whey protein isolate (WPI) and maltodextrin (MD) during spray drying.

- To develop spray drying process to extract *O. stamineus* using ethanol assisted with ultrasonic extraction method.
- To optimize the microencapsulation process and minimizing thermal degradation of *O. stamineus* compound by manipulation the selected parameters.

1.7 Scope of this research

In this study the parameter that will be manipulated during the spray drying microencapsulation are the inlet temperature of air, the size of the nozzle, total solid content, and the ratio of maltodextrin and whey protein. Inlet temperature of air to the spray dryer will be at minimum point of 140 °C to the maximum point of 190 °C. The size of spray dryer nozzle that will be used are 0.5, 1.0, and 2.0 mm. Total solid will be set at 5% and 20%. Initially, the percentage of maltodextrin will be 10% while whey protein is 90%, and the ratio will be changed until the percentage of maltodextrin is 90% and whey protein is 10%. The set of experiment that comes out with the lowest polyphenol degradation is the most optimum for the microencapsulation.

1.8 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 provides a description of each aspect touched in this research project based on different past research or articles that are available in the literature. The description of the main material of this project, *O. stamineus* was discussed by many researchers. The flavonoid content, extraction method, microencapsulation method and the optimization which done by them also was reviewed.

Chapter 3 gives a review of the method on carrying out from the early stage of experiments to the end. It goes from extraction, microencapsulation, drying process and analysis of the samples for total solid, particle size distributions and also UPLC analysis after spray drying.

Chapter 4 is devoted to the analysis of the data obtained from the samples. The analysis involved will be total phenolic content, total flavonoid content, and the antioxidant activity.

2 LITERATURE REVIEW

2.1 Overview

This chapter literally discussed and reviewed on the previous studies done by the other researchers on the *O. stamineus* characteristics, the microencapsulation techniques, the spray drying method. The optimization protocol of various substance also have been studied so that it could be applied for this research project.

2.2 Introduction

Orthosiphon stamineus is known locally in Malaysia as Misai Kucing. *O. stamineus* is also found in other countries such as Thailand, Indonesia and Europe. In these places, Misai Kucing is also known as Yaa Nuat Maeo, Rau Meo or Cay Bac (Thailand); Kumis Kucing or Remujung (Indonesia); Moustaches de Chat (France); or Java Tea (Europe). This herbal plant is a popular traditional folk medicine which widely used in Southeast Asia for the treatment of a wide range of diseases. In Indonesia, it is used to treat rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorders, gonorrhea, syphilis, renal calculus and gallstones; in Vietnam for urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice and biliary lithiasis; and in Myanmar to alleviate diabetes and urinary tract and renal diseases (Ali et al., 2011). From its popularity and demonstrated effectiveness, phytochemical and pharmacological studies have been conducted since the 1930's, and highly-oxygenated isopimarane- type diterpenes, orthosiphols were reported, together with monoterpenes, triterpenes, saponins, flavonoids, hexoses, organic acids, rosmarinic acid, chromene, and myo-inositol (Ezuka et al., 2000)

2.3 Polyphenol content in *O. stamineus*

Previous scientific studies revealed that extract of *O. stamineus* contained various terpenoids, polyphenols and sterols (Tezuka et al., 2000) leading to various activities such as antibacterial, antifungal, antimicrobial and antitumor. The flavonoids are a diverse group of polyphenolic compounds widely distributed in the plant kingdom and over 4000 structurally unique flavonoids have been identified in plant sources (Patel, 2010). The total flavonoids content was found to be high in the hexane extract

and total phenols in methanolic extract (Tilton, 2014). The therapeutics effects of *O. stamineus* have been described mainly to its polyphenol, which has enzyme inhibition and antioxidant activity (Hossain & Rahman, 2011).

Misai Kucing is famous for its flavonoids which are bioactive due to the presence of phenolic compounds in their structures. Twenty phenolic compounds have been isolated from *Orthosiphon stamineus*, including nine lipophilic flavones, two flavonol glycosides and nine caffeic acid derivatives, such as rosmarinic acid and 2,3-dicaffeoyltartaric acid. The above mentioned caffeic acid derivatives appear to be the most abundant polyphenols in aqueous methanol extracts and the polymethoxylated flavones predominate (Ho et al, 2014).

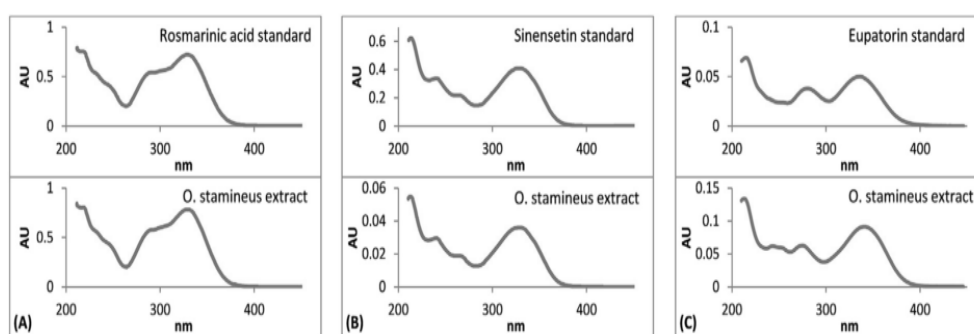


Figure 2-1 Spectra for rosmarinic acid, sinensetin and eupatorin from standard and *O. stamineus* extract. (Source: Assessment of phenolic compounds stability and retention during spray drying of *Orthosiphon stamineus* extracts, 2013)

2.4 Extraction of *O. stamineus*

Ultrasonic-assisted extraction (UAE) with ethanol was used to extract the compounds responsible for the antioxidant activities of Misai Kucing (*Orthosiphon stamineus*) (Ho et al., 2014). The extraction of phenolic compounds was traditionally carried out by using Soxhlet extraction. However, the extraction operated at high temperature for a long time might degrade the extracted compounds, and it also has a high energy cost (Kuo et al., 2013). Ultrasonic-assisted extraction (UAE) is an alternative extraction process that can decrease extraction time and increase extraction yield in many plants. It has mechanical energy with a frequency higher than 18 kHz which is the upper limit that human typically hear. Ultrasonic with frequency more than 1 MHz and power less than 1 W/cm² is used for food and medical diagnosis, while ultrasonic with frequency between 20 to 100 kHz with power more than 5 W/cm² is used for food processing (Prommajak, Surawang, & Rattanapanone, 2014). This extraction method is one of the most simple, low cost extraction systems and can be

operated rapidly in a broad range of solvents for large-scale preparations suited for industrial purposes and this extraction method has attracted much interest in the extraction of bioactive substances from plant materials.

2.5 Microencapsulation of Polyphenol

In the food industry, microencapsulation involves the incorporation of ingredients, polyphenols, volatile additives, colors, enzymes, and bacteria in small capsules to stabilize, protect, and preserve them against nutritional and health losses. In addition, microencapsulation can simplify the food manufacturing processes by converting liquids to solid powders and decreasing production costs (Mahdavi, Jafari, Ghorbani, & Assadpoor, 2014). Microencapsulation of polyphenols from *O. stamineus* using either WPI or maltodextrin as encapsulating agents has successfully reduced polyphenol degradation during spray drying (Pang et al, 2014). The application of microencapsulation technique will be applied to solve this problem with the purpose to produce capsule with high nutritional value for the consumers. Thus, this will increase the health of the consumers for taking the nutraceutical capsule with the highest quantity and quality of bioactive compounds that has the therapeutic effect to treat illness (Ling, 2013).

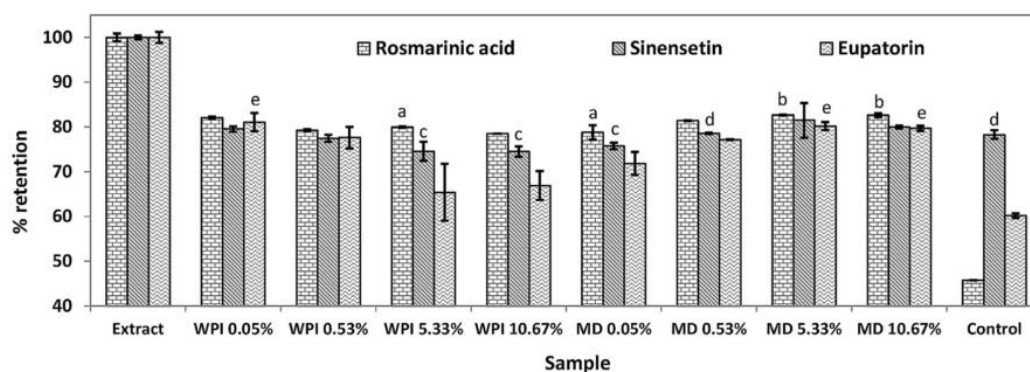


Figure 2-2 Retention of rosmarinic acid, sinensetin and eupatorin using different encapsulation strategies. Means of three replicates followed by at least one same letter are not significantly different ($P > 0.05$) (Pang et al, 2014)

2.6 Spray drying

Spray drying, a leading technology in the food industry, is the most commonly used microencapsulation method for food ingredients. This technique is a well-known

process suitable for drying materials due to the very short heat contact time and the high rate of evaporation resulting in high quality, stable, functional, and low moisture content products (Vict et al, 2013). Microencapsulation can potentially offer numerous benefits to the food ingredients being encapsulated. Handling and flow properties can be improved by converting a liquid to solid encapsulated form. The microencapsulation procedure protects hygroscopic materials from moisture and maintains the stability of ingredients that are volatile or sensitive to heat, light, or oxidation.

The efficiency of encapsulation could be improved with the right selection of spray-drying parameters, including inlet and outlet drying air temperatures, inlet feed temperature, atomization type and conditions, drying air flow rate, and humidity and powder particle size (Mahdavi et al., 2014). The ideal spray- drying conditions are a compromise between high air temperature, high solid concentration of the solution, and easy pulverization and drying without expansion and cracking of final particles.

2.7 Optimization of microencapsulation

The important issues when dealing with extraction of botanical and herb products is the effect of the extraction process on the nutritional or active medicinal components, including their toxicity and the residues of solvent. This point is applicable for both the researcher and herbal and pharmaceutical manufacturing company. Research on optimizing the extraction of *O. stamineus* has been done on the effect of SC-CO₂ on extraction yield and cytotoxicity of *O. stamineus* leaves extracts. In their work, an attempt was made to optimize the different extraction conditions using supercritical fluid extraction method to obtain the maximum yield of the extract of *O. stamineus* leaves with potent antioxidant activity (Al-Suede et al., 2014).

Optimization of phenolic extraction from *Orthosiphon stamineus* was carried out in a study by investigating the effects of ethanol concentration (0-100%, v/v), extraction time (60-300 min) and extraction temperature (25-65°C) on the phenolic recovery using single factor experiment. Total phenolic content (TPC), total flavonoid content (TFC) and condensed tannins content (CTC), used for determination of phenolic compounds while 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical-scavenging capacity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity were used for assessing the antioxidant capacities of crude extract and from the results of this study; as a function of extraction temperature, all antioxidant compounds

assays (TPC, TFC and CTC) were negatively correlated with antioxidant capacities (ABTS and DPPH) (Chew et al, 2011).

Table 2-1 Correlation coefficients between assays under the influence of extraction temperature
(Chew et al, 2011)

Assay	TPC	TFC	CTC	ABTS
TFC	0.993*			
CTC	0.999**	0.994**		
ABTS	-0.947*	-0.973*	-	0.958*
DPPH	-0.942*	-0.948*	-	0.898*

*p<0.05

** p<0.001.

2.8 Optimization through Response Surface Methodology (RSM)

According to (Ho et al., 2014), Optimization of the extraction factors was performed using response surface methodology (RSM), which enables evaluation of independent variables and of any interactions with dependent variables. In their research, they applied (RSM) to optimize four independent variables: ethanol concentration (%), amplitude (%), duty cycle (W/s) and extraction time (min). It should be mentioned that several authors have reported concerns over the potential of ultrasonic cavitation to produce free hydroxyl radicals that might cause phenolic degradation. Compared with a one-factor-at-a-time design, which is adopted most frequently in the literature, the experimental design and RSM were more efficient in reducing the number of experimental runs and time needed for investigating the optimal conditions for extraction (Kuo et al., 2013). The RSM is a dynamic and foremost important tool of Design of Experiment (DOE) where in the relationship between process output(s) and its input decision variables, it is mapped to achieve the objective of maximization or minimization of the output properties. RSM was successfully applied for prediction and optimization of cutting parameters (Soleymani Yazdi & Khorram, 2010).

3 MATERIALS AND METHODS

3.1 Overview

The purpose of the research is to achieve the least phenolic degradation after the sample of *O. stamineus* being extracted, microencapsulated, and spray dried through the optimization using RSM. The list of chemicals are discussed in section 3.2, following by the methods that are going to be applied, including the extraction, microencapsulation, spray drying, and the analysis of the data obtained.

This research is partially based its finding through parameters research method because this permits a flexible and iterative approach. For the data gathering, the choice and design of methods are constantly modified because it will try to find and develop theories that will explain the relationship of one variable with another variable through qualitative elements in research.

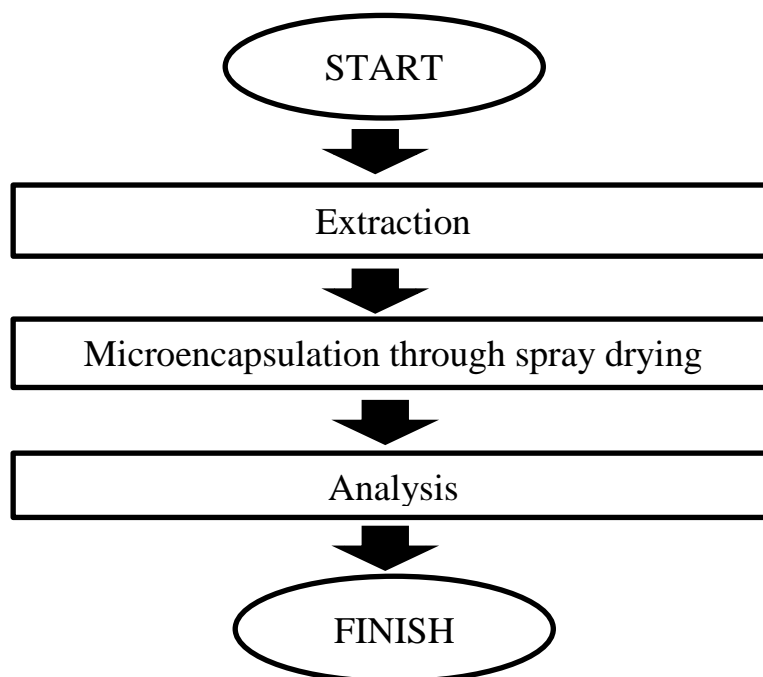


Figure 3-1 Overall process of the experiment

3.2 Chemicals

Main material of this research project, *O. Stamineus* powder will be bought from local supplier. Chemicals like Ethanol, Acetonitrile UPLC grade, Trifluoroacetic Acid, Sodium Hydroxide, Sodium Nitrate, Aluminium Hexachloride, Folin-ciocalteu reagent

are purchased from chemical supplier such as Sigma-Aldrich, Fisher Scientific, Merck, and Fluka. All of these chemical have purity 99.9 mol%. 5 kg of Maltodextrin DE-10 purchased from San Soon Seng Food Industries and lactose-free whey protein isolate powder is purchased from Ultimate Nutrition.

3.3 *Ultrasonic-assisted extraction of O. stamineus*

O. stamineus extracts prepared using ultrasonic-assisted extraction at 50 °C for 90 min at 45 kHz. The extraction time of 90 min was chosen from the initial study on the effect time to the concentration of phenolic compounds, which indicate 90 min as optimum extraction time (Pang et al., 2014). Total of 5.5 g of O. stamineus powder added to 100 ml of 70% ethanol (8 wt.%). The aqueous ethanol will enable a simultaneous extraction of both hydrophilic (hydroxylated, i.e. rosmarinic acid) and lipophilic (methoxylated, i.e. sinensetin and eupatorin) phenolic compounds (Pang et al., 2014). The supernatant will then be separated from the residue by filtration using 0.65µm membrane filter 1 filter. Extract will be concentrated by evaporating out excessive methanol from the extract at 40 °C in rotary evaporator. The total solid content for each sample will be determined by evaporating the liquid from 5 ml solution completely in an oven.

3.4 *Microencapsulation of polyphenol*

The extracts will be encapsulated by two types of wall material which are whey protein isolate and maltodextrin with a dextrose equivalent of 10. WPI and MD powder with amount of 20g each respectively is then added to distilled water, make the final volume of the solution to 100ml to produce 20 w.t% of encapsulating agent. With the temperature of 40 °c, the solution be stirred continuously by using a magnetic stirrer. 10, 5, and 0.5 w.t% of those encapsulating agent then mixed with O. stamineus extract and stirred continuously using magnetic stirrer at 40 °c. The polyphenols retention of WPI and maltodextrin encapsulated powder at various concentrations ranging from 0.05 to 10% were compared to the initial solution to assess the level of polyphenols preservation. The initial solution and the dried powder were examined for their rosmarinic acid, sinensetin and eupatorin content using UPLC.

3.5 Spray drying

A lab scale spray dryer (Lab Plant SD06A, UK) will be used to spray dry the resultant solution. Atomizer with nozzle size of 1.0mm fitted on the spray dryer and air velocity of about 4.1 m/s shall be set constant throughout the experiment. For the purpose of manipulating the temperature parameter, inlet air temperature is to be set at temperature of 140 °C, 165 °C and 190 °C, and this can be carried out by using proportional-integral-derivative controller. Schott bottle which attached at the bottom of cyclone separator will store the collected dried powder samples. In order to attain fair and just comparison, similar setup of experiment used for each test.

3.6 Particle Moisture Content

The moisture content from the spray dried powder was determined using a moisture analyzer (AND MS-70, Japan). Initially, about 1 g of powder sample was placed on the heating pan of the moisture analyser. The moisture content evaporates as a result of continuous heating, and the experiment stopped automatically once the mass of the sample attained a constant value.

3.7 Ultra-Performance Liquid Chromatography

The analysis was done by using Ultra Performance Liquid Chromatography (UPLC). Before the analysis was done, the mobile phases were prepared. UPLC analysis was performed by using Water Acquity UPLC[®] system equipped with photodiode array detector and connected to a computer running Waters Empower 2[®] software. Acquity UPLC HSS T3 C18 Column (2.1 x 75 mm, 1.8 µm inner diameters) was used. The mobile phases consisted of acetonitrile: trifluoroacetic acid (20: 0.001); ultrapure water: trifluoroacetic acid (20:0.001). The temperature was maintained at 30°C, with injection volume of 2 µl and flow rate of 0.170 ml/min. The peaks were detected at 340 nm and identified by standard substances. The reference compounds used as markers were Rosmarinic acid and Sinensetin. The external standard method was used for the UPLC quantification. The results were reported as percent of dry powder weight.

3.8 *Analysis of Polyphenol Content*

The total solid content from *O. stamineus* extract was determined by evaporating the liquid from 5 ml solution completely in an oven. Moisture content for all dried powder samples is determined using a moisture analyser, and the water content is subtracted during preparation of solution for UPLC analysis of polyphenol after spray drying. The same dry weight of solid (bioactive compounds) is set for the initial solution (extract) and after drying the solution to ensure a fair comparison of polyphenol retention. The predetermined amount of dried powder was dissolved in 60% aqueous methanol with aid of vortex mixer to ensure dissolution of less polar compounds. The stock solution of rosmarinic acid (10 mg/ml) was prepared in methanol, whereas eupatorin (10 mg/ml) and sinensetin (5 mg/ml) were dissolved in DMSO. The three analytical standards were further diluted until 0.08 mg/ml to develop an eight points calibration curve. Qualitative and quantitative determinations of *O. stamineus* extract major constituents (rosmarinic acid, sinensetin and eupatorin) were performed on a Waters Acquity UPLC H-Class (Milford, MA) fitted with Acquity UPLC HSS T3 column (2.1 x 75 mm, 1.8 mm) and an Acquity UPLC HSS T3 VanGuard column guard (2.1 x 5 mm, 1.8 mm). The UPLC system is equipped with photodiode array detector and connected to a computer running Waters Empower 2 software. The mobile phase consists of solvent A:water:TFA (20:0.001; v/v) and solvent B: ACN:TFA (20:0.001; v/v) and the following gradient elution: 0e2.0 min, 26% B; 2.0e3.9 min, 26e50% B; 3.9e6.9 min, 50e95% B and finally washing the column with 95% B for 0.6 min and reconditioning the column with 26% B isocratic for 1.4 min. The temperature was maintained at room temperature (24 C), with injection volume of 2 ml and flow rate at 0.17 ml/min. The sample was filtered with 0.2 mm PES membrane filter before injected to the UPLC system. The peaks for rosmarinic acid (3.10e3.30 min), sinensetin (5.50e5.60 min) and eupatorin (5.65e5.75 min) were detected at 340 nm.

To measure particle size distribution of spray dry powder laser diffraction particle size analyzer (Malvern 2000 mastersizer, Malvern Instruments Co., Worcestershire, UK) equipped with an automated dry powder dispersion unit (Scirocco 2000) is to be used. The particle size distribution is characterized by the volume weighted mean.

3.9 Optimization and statistical Analysis

RSM is going to be applied for the optimization protocol for this study. Independent variables of spray dryer temperature, and WPI/MD percentage are to be optimized using this method. RSM will indicate areas in the design region where the process is likely to give desirable results. Simultaneous consideration of multiple responses involves first building an appropriate response surface model for each response and then trying to find a set of operating conditions that in some sense optimizes all response or at least keeps them in desired ranges.

A quadratic model was used to express the responses as a function of independent variables, where A and B are coded values of temperature and wall-to-core ratio. For statistical analysis, each experiment will be repeated in triplicates. Analysis of variance (ANOVA) will be performed by using the data analysis tools in Microsoft Excel 2010, and a least significant difference (LSD) test will used to compare the means with a confidence interval of 95%.

The adequacy of the model was checked by calculating the R^2 and adjusted- R^2 . The numerical and graphical optimization techniques of the Design-Expert software were used for the simultaneous optimization of the multiple responses. The desired goals for each variable and response were chosen. All of the independent variables were kept within range, while the responses were either maximized or minimized.

4 Result and Discussion

4.1 Ultrasonic Assisted Extraction

The grinded powder of *O. stamineus* was extracted to break the cell wall of the plant by shear forces and the active constituents of the plant was observed to be leaching out to the surrounding solvents, ethanol for further mixing. After 90 minutes of extraction done with temperature 50 °C, the extracted solutions turns brownish green colour and a large amount of precipitate was observed in the sealed Erlenmeyer flasks. The precipitate colour was dark green or dark brown. 70% ethanol was best suited solvent to be used in ultrasonic extraction as previous researches had supported that extraction process using 70% ethanol was able to obtain the highest value of antioxidant activity among the solvents studied.

4.2 Moisture Content Test Result

Moisture content of the spray-dried product is an important parameter because it determines whether one needs to adopt post drying processing or not. The spray dry powders obtained containing low moisture content which supporter by Master, (1991) that spray drying process able to produce high quality powders with low moisture content. The results revealed that the moisture content of the spray dried powder reduced with the increased of the total solid contents of the WPI and MD. This is due to the reduction of the total water content in the sample as the solid concentration increased despite exposing to heating when mixing. Following Figure 4-1 and Figure 4-2 are the residual plot of moisture content and predicted vs actual moisture content plot respectively.

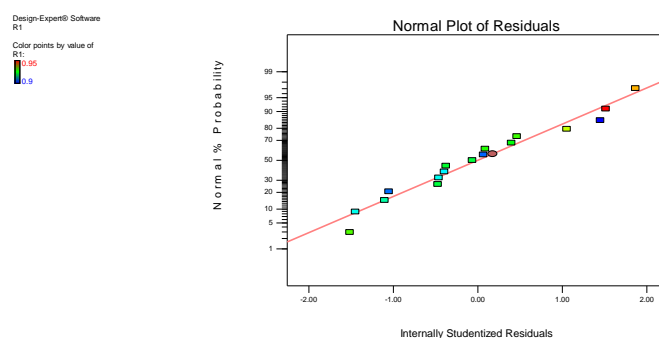


Figure 4-1 Residual plot of moisture content

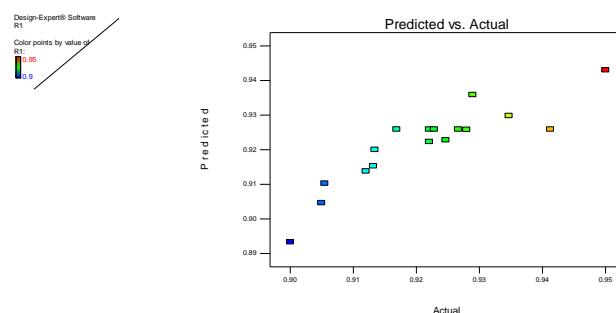


Figure 4-2 Predicted vs actual moisture content

The moisture content percentage is being studied on all parameters. It is due to the undeniable effect of moisture towards the quality of the sample. Lower moisture content prolongs the effect of longer shelf life of most substances including the O. stamineus extract itself.

4.3 Total Solid Content Result

The effects of the total solid content to the degradation of the polyphenol were examined by comparing the microencapsulaton of WPI and MD with various concentrations or the wall to core ratio. The total solid content test shows the trend of the proper comparison by dissolving the same solid content. A comparative amount of powders were dissolved as shown in Table 4-1. Increase in the total solid contents leading to a higher protein denaturation at temperature above 100 °C.

RUN	Temperature (°C)	TSS solid (%)	Wall to core ratio	Total solid content
1	165	12.5	10.5	0.92
2	165	12.5	10.5	0.92
3	140	12.5	16	0.90
4	190	20	10.5	0.91
5	165	12.5	10.5	0.94
6	190	12.5	16	0.93
7	165	20	5	0.95
8	140	5	10.5	0.90
9	190	12.5	5	0.92
10	140	12.5	5	0.91
11	165	5	16	0.93
12	165	12.5	10.5	0.92
13	165	5	5	0.91
14	190	5	10.5	0.93
15	165	12.5	10.5	0.93
16	140	20	10.5	0.91
17	165	20	16	0.92

Table 4-1 The total solid content for different wall to core ratio

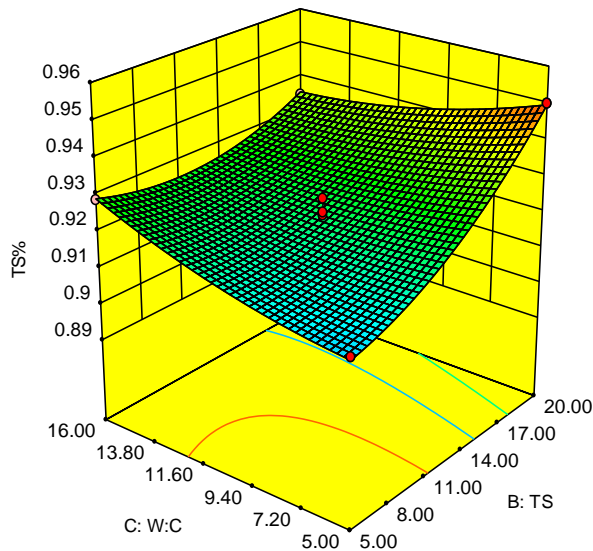


Figure 4-3 Total solid in powder vs total solid in liquid and wall to core ratio

Plot in Figure 4-3 shows the effect of TSS and wall to core ratio on solid content. The increase in wall to core ratio of the microencapsulation results in the increasing moisture content. The moisture content of the encapsulated sample would also become higher with the increasing total solid content.